U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership

AADAP
Aquatic Animal Drug Approval Partnership Program

DRUG RESEARCH INFORMATION BULLETIN

DRIB No. 34
May 2013

The Influence of Life Stage, Water Temperature, and Eugenol Concentration on the Sedation of Rainbow Trout with AQUI-S®20E (10% Eugenol)

Dan Carty*, Jim Bowker, Molly P. Bowman, and Niccole Wandelear

U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana 59715, USA

AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) is a candidate for U.S. Food and Drug Administration (FDA) approval for use as an immediate-release sedative in fisheries research, fisheries management, and fish culture. As part of the ongoing AQUI-S®20E approval process, efficacy trials have been conducted on a variety of freshwater-reared finfishes (e.g., Bowker et al. 2013a, 2013b, and 2013c). In addition, we conducted a pilot trial to investigate the influence of fish life stage, water temperature, and eugenol concentration on the times required for freshwater-reared Rainbow Trout *Oncorhynchus mykiss* to become handleable and recover from handleable when sedated with AQUI-S®20E.

Methods

Testing facility, test fish, and test article.—The trial was conducted between October 20 and November 11, 2011, at the U.S. Fish and Wildlife Service (FWS) Bozeman Fish Technology Center, Bozeman, Montana USA. Test fish were Rainbow Trout, which had been obtained as eyed eggs from the FWS Ennis National Fish Hatchery, Ennis, Montana USA. The test article was AQUI-S®20E, which had been obtained from AQUI-S New Zealand, Ltd.

Experimental design and procedures.—The experimental design was a Model I (fixed effects) 2 × 2 × 2 factorial (Zar 2010; Table 1). Factor A was Rainbow Trout life stage (juvenile or adult); factor B was water temperature (10 or 16ºC); and factor C was eugenol concentration (25 or 75 mg per L). The design incorporated eight treatment groups, and there were 30 fish per group. Consequently, 240 fish were used in the trial.

For each treatment group, a bulk tub of AQUI-S®20E solution (151 L) was prepared, and three 125-mL samples of the solution were collected and analyzed by spectrophotometry to confirm eugenol concentration. Aliquots of bulk solution were used to fill individual sedation containers, and fish were individually sedated in these containers under static conditions. One juvenile fish was sedated per container before the sedative solution (2 L) was discarded and the container refilled. However, five adult fish were individually sedated per container before the sedative solution (23 L) was discarded and the container refilled.

A fish was determined to be handleable when it lost equilibrium and the ability to swim, could easily be caught by and held in hand, and measured for length and weight with little or no movement. When a fish became handleable, it was removed from the sedative solution, measured for length and weight, and transferred to a container of fresh, flowing water. A fish was determined to be recovered when it regained equilibrium, resumed normal swimming behavior, and avoided obstacles (e.g., a net handle) placed in its path. Times to handleable and recovery were recorded for each fish, and fish behavior was evaluated during sedation and recovery. Following recovery, fish were placed in a holding tank plumbed with fresh, flowing water and monitored for survival for 24 h. No fish was used more than once during the trial. Water temperature and dissolved oxygen concentration were measured in each sedation container and recovery tank.

Statistical analysis.—Time-to-handleable and time-to-recovery data were analyzed separately. In each analysis, we used general linear models in SAS JMP 8.0 to (1) identify the main effects (A, B, and C) and interaction effects (A × B, A × C, B × C, and A × B × C) that significantly ($P < 0.05$) influenced the desired endpoint (handleable or recovered) and (2) determine how much of the overall variation in time to handleable or recovery was explained by the significant effects. All main and interaction effects were treated as fixed, categorical variables. Before the time-to-handleable and time-to-recovery data were analyzed, the Box-Cox transformation (Osborne 2010) was used to correct for or minimize problems with nonnormal distributions and heterogeneity of variances.

Results

Time to handleable.—Time to handleable ranged from a low of 0.6 ± 0.0 min (mean ± 95% CI) for juvenile fish tested at 16ºC and 75 mg per L to a high of 1.8 ± 0.2 min for juvenile fish tested at 10ºC and 25 mg per L (Figure 1). Additionally, there was an increasing trend in time to handleable from (1) fish tested at 16ºC and 75 mg per L to (2) fish tested at 10ºC and 75 mg per L to (3) fish tested at 16ºC and 25 mg per L to (4) fish tested at 10ºC and 25 mg/L (Figure 1).

The main effects of eugenol concentration and water temperature were the only variables that significantly influenced time to handleable. These two variables explained

*Corresponding author: dan_carty@fws.gov

79% of the overall variation observed in time to handleable, with eugenol concentration accounting for 70% and water temperature accounting for 9%. In contrast, neither the main effect of life stage nor any of the interaction effects among life stage, water temperature, and eugenol concentration significantly influenced time to handleable.

Time to recovery.—Time to recovery ranged from a low of 3.6 ± 0.3 min (mean ± 95% CI) for juvenile fish tested at 16ºC and 25 mg per L to a high of 6.7 ± 0.5 min for adult fish tested at 10ºC and 75 mg per L (Figure 2). The main effects of life stage, water temperature, and eugenol concentration, as well as the interaction between water temperature and eugenol concentration significantly influenced time to recovery; however, these four variables explained only 41% of the overall variation observed in time to recovery. Separately, life stage (16%) and eugenol concentration (15%) accounted for more of the overall variation observed in time to recovery than did water temperature (6%) or the interaction between water temperature and eugenol concentration (4%).

Juvenile fish tended to recover more quickly than adult fish (Figure 2). One exception to this was that adult fish tested at 16ºC and 25 mg per L recovered within the range of mean times observed for juvenile fish (Figure 2). Also, treatment groups with shorter mean times to handleable tended to have longer mean times to recovery (Figure 3). The one exception to this result was adult fish tested at 10ºC and 25 mg per L, which had the second-longest mean time to handleable and the second-longest mean time to recovery (Figure 3).

Fish mortality and behavior.—No fish died during the trial. In the 25 mg per L treatment groups, 3% (three juveniles and one adult) of the fish tested exhibited headshaking or gill coughing behavior. In contrast, in the 75 mg per L treatment groups, 44% (28 juveniles and 25 adults) of the fish tested exhibited headshaking, coughing, or slight agitation behavior. These types of behaviors lasted only a few seconds, ceased as fish became sedated, and appeared to have no adverse effects on the fish through the 24-h postrecovery observation period. During recovery from sedation at 25 or 75 mg per L, all fish appeared to behave normally.

Eugenol concentration.—The analytically verified mean ± SD eugenol concentrations from the 25 mg/L solutions ranged from a low of 23.1 ± 0.2 mg per L (-8% of target) to a high of 25.2 ± 0.2 mg per L (+1% of target; Table 1). In the 75 mg per L treatment groups, the mean eugenol concentrations ranged from a low of 69.3 ± 0.2 mg per L (-8% of target) to a high of 74.9 ± 0.5 mg per L (±0% of target; Table 1).

Water temperature and dissolved oxygen concentration.— In the 10ºC treatment groups, mean water temperatures for sedation and recovery ranged from 10.1 to 10.9ºC. In the 16ºC treatment groups, mean water temperatures for sedation and recovery ranged from 16.2 to 16.6 ºC. Mean dissolved oxygen concentrations were slightly greater in the 10ºC treatment groups (range of means, 8.6 to 9.9 mg per L) than in the 16ºC treatment groups (range of means, 7.3 to 8.2 mg per L).

Discussion and Conclusions

The efficacy of AQUI-S®20E and other fish sedatives can be influenced by a variety of biotic and abiotic factors. Other than sedative concentration, Burka et al. (1997) listed species, life stage, length, weight, lipid content, body condition, health status, water temperature, pH, and dissolved salts as possibilities. Given the conditions under which the current trial was conducted, we found that eugenol concentration had the most influence on time to handleable and explained a large portion of the overall variation observed in handleable times. In contrast, life stage and eugenol concentration equally influenced time to recovery but explained less than half of the overall variation observed in recovery times. Also, we noted a tendency for juvenile fish to recover faster than adult fish and a weak inverse relation between time to handleable and time to recovery. The data generated in the current trial were accepted by FDA as supporting approval of AQUI-S®20E for use in the U.S. as an immediate-release fish sedative.

Acknowledgments

Dave Erdahl, USFWS AADAP Program, reviewed this bulletin.

References

Bowker, J., D. Carty, M. P. Bowman, and N. Wandelear. 2013a. Use of AQUI-S®20E and BENZOAK® to sedate rainbow trout, cutthroat trout, and brown trout to handleable. *Drug Research Information Bulletin No. 30*. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana USA.

Bowker, J., D. Carty, and N. Wandelear. 2013b. Use of AQUI-S®20E and BENZOAK® to sedate walleye, yellow perch, common carp, and fathead minnow to handleable. *Drug Research Information Bulletin No. 31*. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana USA.

Bowker, J., D. Carty, and N. Wandelear. 2013c. Use of AQUI-S®20E and BENZOAK® to sedate sunshine bass, blue catfish, and Nile tilapia to handleable. *Drug Research Information Bulletin No. 32*. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana USA.

Burka, J. F., K. L. Hammel, T. E., Horsberg, G. R., Johnson, D. J. Rainnie, and D. J. Spears. 1997. Drugs in salmonid aquaculture – a review. *Journal of Veterinary Pharmacology and Therapeutics* **20:333-349**.

Osborne, J. W. 2010. Improving your data transformations: applying the Box -Cox transformation. *Practical Assessment, Research, & Evaluation* **15: Article No. 12 (9 pages)**.

Zar, J. H. 2010. *Biostatistical Analysis*. Prentice Hall, Inc., Upper Saddle River, New Jersey USA.

Table 1. Rainbow Trout tested at each combination of life stage, water temperature, and eugenol concentration.[1] (na = not applicable)

Fish life stage, nominal water temperature, and nominal eugenol conc.	Treatment group code	Date tested	Sample size (*n*)	Mean ± SD length (mm)	Mean ± SD weight (g)	Mean ± SD measured eugenol conc. (mg per L)
Juvenile 10ºC						
25 mg/L	J-10-25	Nov 03	30	182 ± 17	68 ± 17	25.2 ± 0.2
75 mg/L	J-10-75	Nov 02	30	170 ± 25	58 ± 22	71.1 ± 0.2
Juvenile 16ºC						
25 mg/L	J-16-25	Oct 20	30	157 ± 22	44 ± 17	24.9 ± 0.1
75 mg/L	J-16-75	Oct 20	30	165 ± 24	50 ± 18	74.9 ± 0.5
All juvenile fish	na	na	120	168 ± 24	55 ± 20	na
Adult 10ºC						
25 mg/L	A-10-25	Nov 09	30	412 ± 30	1,003 ± 224	23.3 ± 0.1
75 mg/L	A-10-75	Nov 09	30	400 ± 21	920 ± 162	69.3 ± 0.4
Adult 16ºC						
25 mg/L	A-16-25	Oct 27	30	395 ± 29	860 ± 205	23.1 ± 0.2
75 mg/L	A-16-75	Oct 27	30	395 ± 33	800 ± 207	70.0 ± 0.4
All adult fish	na	na	120	400 ± 29	896 ± 212	na

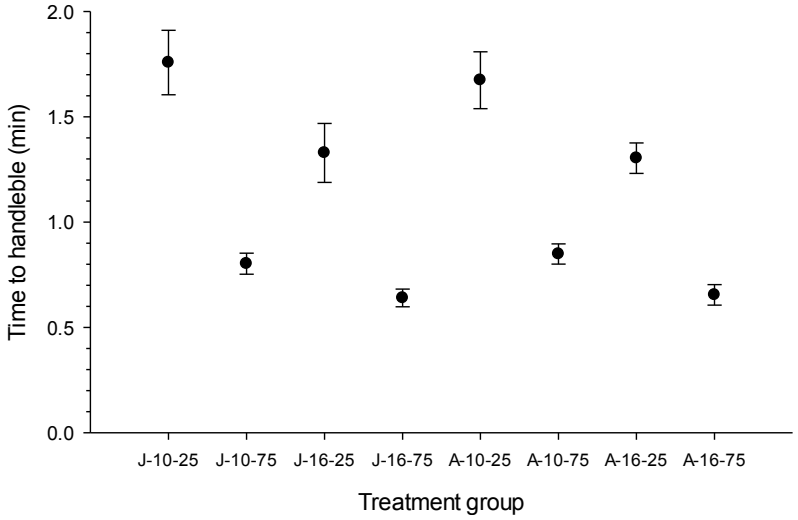

Figure 1. Mean ± 95% CI time to handleable for Rainbow Trout tested at each combination of life stage, water temperature, and eugenol concentration (n = 30 fish per treatment group).[1]

[1]Treatment group codes: J = juvenile, A = adult, 10 = 10ºC, 16 = 16ºC, 25 = 25 mg per L, and 75 = 75 mg per L.

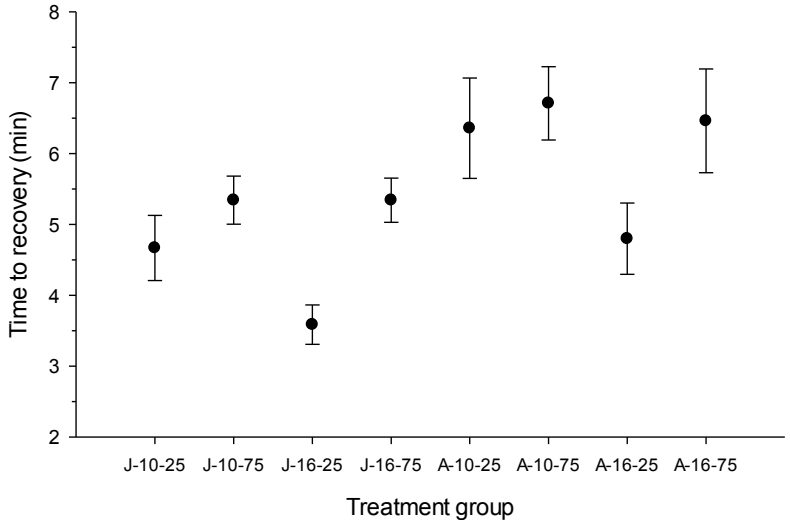

Figure 2. Mean ± 95% CI time to recovery for Rainbow Trout tested at each combination of life stage, water temperature, and eugenol concentration (n = 30 fish per treatment group).[1]

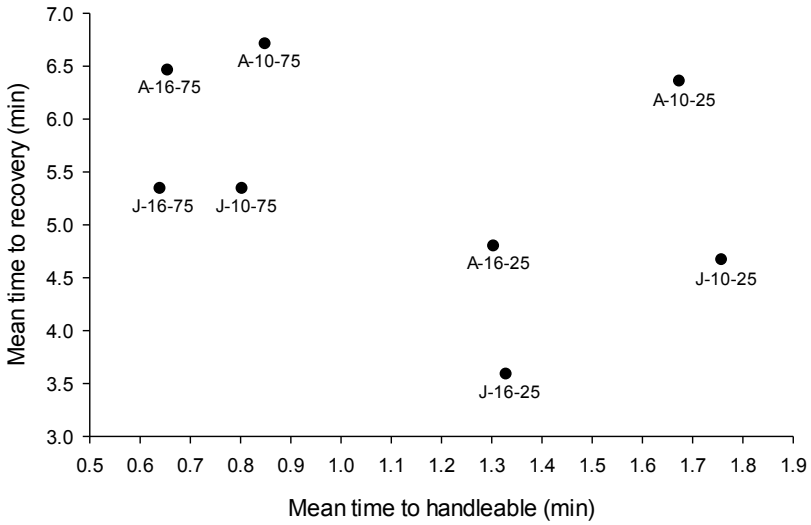

Figure 3. Mean time to recovery plotted against mean time to handleable for Rainbow Trout tested at each combination of life stage, water temperature, and eugenol concentration (n = 30 fish per treatment group).[1]

[1]Treatment group codes: J = juvenile, A = adult, 10 = 10ºC, 16 = 16ºC, 25 = 25 mg per L, and 75 = 75 mg per L.